

# *Transgenic cereals: current status and future prospects*

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Jim M. Dunwell

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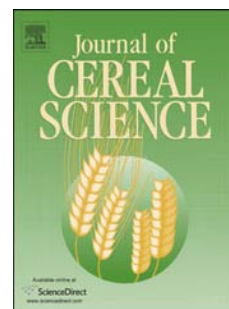
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- The current commercial status of GM cereal is described
- Research on input (agronomic characteristics) and output (grain quality etc) traits is reported
- Data from global field trials are summarised
- Research trends from examination of patent databases are reported
- Public perception and regulatory issues are discussed

# Transgenic cereals: current status and future prospects

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## **Abstract**

This review summarises the history of transgenic (GM) cereals, principally maize, and then focuses on the scientific literature published in the last two years. It describes the production of GM cereals with modified traits, divided into input traits and output traits. The first category includes herbicide tolerance and insect resistance, and resistance to abiotic and biotic stresses; the second includes altered grains for starch, protein or nutrient quality, the use of cereals for the production of high value medical or other products, and the generation of plants with improved efficiency of biofuel production. Using data from field trial and patent databases the review considers the diversity of GM lines being tested for possible future development. It also summarises the dichotomy of response to GM products in various countries, describes the basis for the varied public acceptability of such products, and assesses the development of novel breeding techniques in the light of current GM regulatory procedures.

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27 **Highlights**

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29 *Keywords:* Genetically modified; Maize; Wheat; Barley

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## 1. Background

On a global basis the cereals wheat, maize, rice, barley and sorghum are grown on almost 700 million hectares and collectively they provide approximately 40% of the energy and protein components of the human diet (Table 1). They therefore represent a vital contribution to food security both at present and also in the future when population growth (Dunwell, 2013) and other social and economic trends will require an approximate doubling of food production by 2050. Specific retrospective and prospective data for wheat yields, based on information from the Wheat initiative ([www.wheatinitiative.org](http://www.wheatinitiative.org)) are given in Table 2. In the words of the G20 Agriculture vice-ministers and deputies report from 2012 “Increasing production and productivity on a sustainable basis in economic, social and environmental terms, while considering the diversity of agricultural conditions, is one of the most important challenges that the world faces today” (<http://www.g20.org/en>) . The UK Secretary of State for the Department for the Environment, Food and Rural Affairs made a major speech on 20<sup>th</sup> June 2013 about the role of GM in the future of agriculture (<https://www.gov.uk/government/speeches/rt-hon-owen-paterson-mp-speech-to-rothamsted-research>), and the European Academies Science Advisory Council has recently published a detailed report on the opportunities of using GM technologies in sustainable agriculture (EASAC, 2013).

Against the background of this need for increased agricultural production, this review will consider the history of genetically modified (GM) or transgenic cereals during the 30 year period since the production of the first GM plants in 1983, before discussing their present status and future potential. Information has been obtained not only from recent scientific



literature but also from analysis of regulatory databases for GM crops, and from the patent literature.

## 2. Methods for production of GM plants

The original method devised for the production of the first GM plants in 1983 depended on the use of the natural bacterial vector *Agrobacterium tumefaciens*. At that time it was assumed that this system could not be applied to cereal species and the emphasis for these crops was focussed on direct gene transfer methods, particularly the “gene-gun” or Biolistics technology. This technology was the first method successfully applied to maize. Since that time, significant improvements have been made to the *Agrobacterium* techniques, and these techniques can now also be applied to cereals. A recent summary of a diverse range of GM techniques is available in Dunwell and Wetten (2012).

These novel technologies include new methods for the design of constructs (Coussens et al., 2012; Karimi et al., 2013), that is the DNA sequences to be introduced and improved methods for DNA delivery. These latter methods include techniques for maize (Kirienko et al., 2012), wheat (Tamás-Nyitrai et al., 2012), rice (Duan et al., 2012b; Wakasa et al., 2012), barley (Holme et al., 2012a), triticale (Ziemienowicz et al., 2012), and tef (*Eragrostis tef*) (Gebre et al., 2013). There is also an improved understanding of the process of regeneration from plant cells in culture (Delporte et al., 2012), an important aspect of any system for high efficiency transformation.

Temporal and spatial stability of transgene expression, as well as well-defined transgene incorporation are additional features to be considered (Bregitzer and Brown, 2013; Kim and An, 2012). Likewise, it is of practical importance that GM lines can be rapidly identified,

both in the laboratory (Chen et al., 2012b; Han et al., 2013b; Hensel et al., 2012; Mieog et al., 2013; Xu et al., 2013a) and under field conditions.

Another objective in many GM research projects is the development of more efficient methods for the introduction of multiple genes. These include the construction of mini-chromosomes in rice (Xu et al., 2012a). Additionally, there has been significant progress with efforts to induce site-specific gene integration (Nandy et al., 2012; Kapusi et al., 2012) and to use GM techniques to suppress selected genes or gene families (Wang et al., 2013b). Some of these techniques are also associated with the new techniques described below in section 5.3.

Immediately following the description of GM plants of tobacco in 1983, the commercial focus became the development of GM maize, as this crop was already hybrid and annual sales of such high-value seed was an established part of the agricultural economy of the USA and elsewhere. In contrast, the other important cereals wheat and rice are self-pollinating crops and the value of seed sales is comparatively low, and any GM variety could in theory, if not in practice, be saved by the farmer for growth in subsequent years. For this reason, there have been several attempts to convert inbreeding species into hybrid crops either through the use of chemical hybridizing agents or via GM technology. One GM approach to the production of male sterility, a necessary component of any hybrid system (Feng et al., 2013), has recently been exemplified in wheat by expressing a barnase gene (Kempe et al., 2013).

In the summaries below, the specific traits incorporated into GM varieties will be divided into those that provide advantages to the farmer/grower, the so-called input traits and those that modify the characteristics of the harvested product, the so-called output traits.

### 3. Input traits

#### *3.1. Herbicide tolerance*

Prior to GM technology herbicides were classified into two categories, either selective, those that killed weeds and not crops, and non-selective, those that killed all plants. The development of selective herbicides, in particular, is a very difficult research challenge that requires an understanding of biochemical targets found only in weeds. Transgenic technology opened the possibility of converting non-selective compounds into selective ones, if a gene conferring resistance could be identified, isolated and then transferred into the crop of interest. The most obvious candidate for this strategy was glyphosate, a widely used selective herbicide marketed by Monsanto. Eventually, a bacterial resistance gene was identified and Monsanto subsequently acquired this technology, the means of introducing this gene into maize, and a company which owned elite maize inbred lines, the target for this technique. This company then had the significant commercial advantage of being able to sell both GM herbicide-tolerant (HT) varieties, and the herbicide in question. This combined approach became highly successful and provided the blueprint for many subsequent commercial programmes in maize and other crops. The second major herbicide resistant trait was that conferring tolerance to glufosinate. The commercial need for companies to be able to market both the herbicide and HT crops containing the gene conferring tolerance led to many conflicts associated with intellectual property rights (IPR) and many mergers and acquisitions. The process of consolidation of IPR began in earnest in August 1996 with AgrEvo's purchase of Plant Genetic Systems (PGS) for \$730 million, made when PGS's prior market capitalization was \$30 million. According to AgrEvo, \$700 million of the

purchase price was assigned to the valuation of the patent-protected trait technologies (ie glufosinate resistance gene) owned by PGS (Pila, 2009). In all such cases it is important to avoid any yield drag associated with the presence of the transgene (Darmency, 2013).

At present most hybrid maize sold in the USA is resistant to one or more herbicides. The availability of such HT crops has provided the farmer with a variety of flexible options for weed control (Brookes and Barfoot, 2013a), despite some problems caused by the development of HT weeds, an issue that has stimulated the development of improved versions of glyphosate resistance genes and also of novel genes encoding resistance to other herbicides such as 2,4-D. In some regions, particularly in sub-Saharan Africa, HT maize has also provided a novel control strategy for hemi-parasitic weeds such as *Striga* (Ransom et al., 2012).

One novel finding in the area of HT crops is that showing the resistance of melatonin-rich GM rice plants to herbicide-induced oxidative stress (Park et al., 2013).

Monsanto also developed a glyphosate tolerant (Roundup Ready<sup>TM</sup>) version of wheat, and carried out successful field tests in the 1990s. Due to concerns about international trade of GM wheat, this project was suspended in 2005, although recently in April 2013 some HT wheat plants carrying the Monsanto CP4 gene for glyphosate tolerance have been discovered growing in a farm in Oregon; their origin is uncertain (Fox, 2013; Ledford, 2013).

### 3.2 . Insect resistance

The second target for GM development, together with herbicide tolerance, was insect resistance, specifically the potential that might be provided by the toxins found in the soil bacterium *Bacillus thuringiensis* (Bt). Various proteins from this bacterium were known to be toxic to a range of insects and had been used widely as sprays in agriculture and forestry since the 1950s. Improvements in molecular biology and microbiology during the 1980s meant that the genes encoding these proteins could now be isolated from various strains of the bacterium and introduced into crops. The first target was the corn borer (*Ostrinia nubilalis*), a lepidopteran pest of maize. Subsequently, other Bt genes were isolated; these provided resistance to other pests including the coleopteran species, corn root worm (*Diabrotica* spp.) (Narva et al., 2013). Present maize varieties sold in the USA have several Bt genes, usually combined with herbicide tolerance (Edgerton et al., 2012); in total there may be eight transgenes in a single variety. Recently the experience obtained from the first billion acres of Bt crops was reviewed (Tabasnik et al., 2013).

Such analysis has several aspects. One of the most important has been the need to prolong the life time of these GM varieties by avoiding the development of resistance in the target insects; the history of many insecticides suggests that resistance will eventually develop after prolonged application of any particular compound. Since the first GM products were marketed there has been advice on the need for refugia, areas of non-GM plants (Tabashnik and Gould 2012). This strategy reduces the incidence of insects carrying a mutant resistance gene in the homozygous state. As this refugia policy was not adopted by some farmers, resistant insects have indeed developed in recent years, and it is now suggested that at least five pests have developed such resistance (Tabasnik et al., 2013). Novel approaches to this issue include the combination of different Bt genes (Edwards et al., 2013), or genes with

different modes of action, and the adoption of seed mixes in which Bt and non-Bt seeds are combined (Carroll et al., 2013; Zukoff et al., 2012).

Another significant environmental concern is the possibility of non-target effects, that is the susceptibility of non-pest beneficial insects to the various insecticidal proteins. This is a key element of all regulatory applications for sale of such products. Recent studies of this topic include those on the effects of Bt rice on a generalist spider (Tian et al., 2012) and thrips (Akhtar et al., 2013), Bt maize on bees (Dai et al., 2012) and other arthropods (Alcantera 2012; Comas et al., 2103), and the effect on aphids of GM wheat expressing a snowdrop lectin (Miao et al., 2011).

There have also been some unexpected beneficial side-effects of insect resistant crops. For example, Bt-expressing corn rootworm resistant maize has been shown to have improved nitrogen uptake and nitrogen use efficiency (Haegele and Below, 2013). These results may lead to improved agronomic practices (Bender et al., 2013). Similarly, increased microbial activity and nitrogen mineralization has also been shown in Bt maize (Velasco et al., 2013). This contrasts with the data of Cotta et al. (2013), Lupwayi and Blackshaw (2013) and Fließbach et al. (2013) who found no differences in the microbial communities from the rhizosphere of GM and non-GM maize, and particularly of Han et al. (2013a) who claim that Bt rice reduced the methane emission flux and the methanogenic archaeal and bacterial communities in paddy soils.

Other approaches to insect resistance include modification of the volatile emissions produced by a plant in order to deter pests or to attract beneficial insects. Such a study of GM maize expressing a terpene synthase gene showed that the costs of constitutive volatile production

outweighed its benefits (Robert et al., 2013). An alternative route is to use plant-derived double-stranded RNA to target the suppression of genes essential for insect survival. This method has been shown to be effective in inhibiting growth of the Western Corn Root Worm (*Diabrotica virgifera*) (Bachman et al., 2013; Bolognesi et al., 2012).

### 3.3. Pathogen tolerance

#### 3.3.1. Fungi

Although there are no commercial GM cereals with pathogen tolerance there has been a great deal of research on this subject, with promising results from both laboratory and field tests, particularly with wheat (<http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf>). Wheat is affected by a number of fungal diseases such as stem rust (*Puccinia graminis*), *Septoria*, *Fusarium*, common bunt (*Tilletia tritici*) and take-all, caused by the fungus *Gaeumannomyces graminis*. Among these diseases, *Fusarium* is probably the most significant, causing crown rot and head blight that result in production of small and stunted grains or no grain at all. Some *Fusarium* strains also produce mycotoxins, compounds which when ingested by humans or animals may cause serious illness. These toxins, which are subject to regulation in the human food chain, can also inhibit the growth of yeast during the fermentation of cereal starch to produce bioethanol. For many years Syngenta worked on the development of a *Fusarium*-resistant wheat but this project was suspended in 2007, also after concerns about exports of GM wheat from the USA. Among the genes that have been shown to provide resistance to this fungus are a bovine lactoferrin gene (Han et al., 2012; Lakshman et al., 2013), an *Arabidopsis thaliana* *NPR1* (non-expressor of PR genes) gene (Gao et al., 2013), a polygalacturonase-inhibiting protein gene from *Phaseolus vulgaris* (PvPGIP) (Ferrari et al., 2012) (see also Janni et al., 2013), a lipid transfer gene from wheat

(Zhu et al., 2012b) and the antimicrobial peptides genes *MsrA2* and *IOR* (Badea et al., 2013).

Results from this latter study showed that T3 generation GM plants had a 53% reduction in

*Fusarium* damaged kernels, and some lines also had a 59% reduction in powdery mildew

susceptibility compared with the non-GM control.

Other GM approaches to achieving mildew resistance in wheat include the use of virus-

induced gene silencing (VIGS) of *Mlo* genes (Várallyay et al., 2012), alleles of the resistance

locus *Pm3* in wheat, conferring race-specific resistance (Brunner et al., 2012). Related studies

on this latter material showed that the mildew-resistant GM lines harboured bigger aphid

populations (*Metopolophium dirhodum* and *Rhopalosiphum padi*) than the non-transgenic

lines (von Burg et al., 2012). These results suggest that wheat plants that are protected from a

particular pest (powdery mildew) became more favourable for another pest (aphids). Other

evidence with the same material comes from a study of plots containing either monocultures

or mixtures of two GM lines (Zeller et al., 2012). It was found that resistance to mildew

increased with both GM richness (0, 1, or 2 *Pm3* transgenes with different resistance

specificities per plot) and GM concentration (0%, 50%, or 100% of all plants in a plot with a

*Pm3* transgene). Additional studies by Zeller et al. (2013) concluded that many genes

providing resistance against fungal pathogens demonstrate a significant cost of resistance

when expressed constitutively. Studies on powdery mildew in barley include one that

examined the effect of modifying the expression of the HvNAC6 transcription factor (Chen et

al., 2013).

Other recent tests have described resistance to take-all in GM wheat lines expressing an

R2R3-MYB gene from *Thinopyrum intermedium* (*TiMYB2R-1*) (Liu et al., 2013b) or a potato

antimicrobial gene (Rong et al., 2013), to *Bipolaris sorokinia* by expression of the related



gene *TaPIMP1* (Zhang et al., 2012d), to *Penicillium* seed rot in lines expressing puroindolines (Kim et al., 2012), and to rust diseases by endogenous silencing of *Puccinia* pathogenicity genes (Panwar et al., 2013) and expression of the *Lr34* durable resistance gene (Risk et al., 2012, 2013) or TaRLP.1 (Jiang et al., 2013b). The recent discovery of the wheat *Sr35* gene that confers resistance to the Ug99 strain of rust (Saintenac et al., 2013) may also provide new GM strategies to combat this disease.

Related results from rice include resistance to rice blast (*Magnaporthe oryzae*) in lines expressing a chimeric receptor consisting of the rice chitin oligosaccharides binding protein (CEBiP) and the intracellular protein kinase region of *Xa21* (Kouzai et al., 2013). Similarly lines expressing the *WRKY30* gene showed improved resistance to rice blast and rice sheath blast (*Rhizoctonia solani*) (Peng et al., 2012), and lines expressing a bacterial  $\alpha$ -1,3-glucanase (AGL-rice) showed strong resistance not only to the two blast pathogens but also to the phylogenetically distant ascomycete *Cochlioborus miyabeanus* (Fujikawa et al., 2012). In maize silencing of a putative cystatin gene (*CC9*) improved resistance to the biotrophic pathogen *Ustilago maydis* (van der Linde et al., 2012)

### 3.3.2. Bacteria

It has been shown recently that silencing of the dominant allele of rice bacterial blast resistance gene *Xa13* by using artificial microRNA technology generates plants highly resistant to this pathogen (Li et al., 2012a). These authors suggest that this approach may provide a paradigm that could be adapted to other recessive resistance genes. In an alternative approach, expression of *TaCPK2-A*, a calcium-dependent protein kinase gene that is required for wheat powdery mildew resistance has been shown to enhance bacterial blight resistance in transgenic rice Geng et al., 2013).

### 3.3.3. Viruses

Projects designed to improve virus resistance in cereals include expression of an artificial microRNA to provide resistance to wheat streak mosaic virus (Fahim et al., 2012), and of a dsRNA-specific endoribonuclease gene to provide resistance to maize rough dwarf disease (MRDD) (Cao et al., 2013). It has been reported that a wheat line with resistance to yellow mosaic virus is expected to be available in the market by 2015 (<http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf>). Related studies in rice include resistance to rice stripe disease (RSD) (caused by rice stripe virus, RSV) by expression of an RNAi construct containing the coat protein gene (CP) and disease specific protein gene (SP) sequences from RSV (Zhou et al., 2012b). A similar strategy was employed to improve resistance to the rice gall dwarf virus (RGDV) (Shimizu et al., 2012b) and rice grassy stunt virus (Shimizu et al., 2013).

### 3.4 Abiotic stress

Following the great commercial success of herbicide tolerant and insect resistant crops, research focus moved to the more difficult subject of tolerance to abiotic stress such as drought, salt tolerance and nitrogen and phosphate deficiency. The first commercial cereal product in this area is the Monsanto GM maize DroughtGard™ variety that expresses *cspB*, an RNA chaperone gene from *Bacillus subtilis* (Castiglioni et al., 2008). This gene, which increases yield under water-limited conditions, is also being incorporated into maize adapted to African conditions, as part of the WEMA project (Water Efficient Maize for Africa). There is a wide range of other approaches that are being tested at present in order to improve the growth of cereals under conditions of abiotic stress (Saint Pierre et al., 2012). For example, wheat over-expressing the 12-oxo-phytodienoic acid gene (*TaOPR1*) significantly enhanced the level of salinity tolerance (Dong et al., 2013). It is thought that this gene acts during episodes of abiotic stress response as a signaling compound associated with the regulation of the ABA-mediated signalling network. It is also reported that barley plants expressing the mitogen activated protein kinase HvMPK4 demonstrated improved tolerance to saline conditions (Abass and Morris, 2013).

Overexpression of a phytochrome-interacting factor-like protein, OsPIL1, in transgenic rice plants promoted internode elongation (Todaka et al., 2012). The data suggested that OsPIL1 functions as a key regulatory factor of reduced plant height via cell wall-related genes in response to drought stress and may be useful in improving plant regrowth under such conditions.

GM rice overexpressing the transcription factor OsbZIP16 exhibited significantly improved drought resistance, which was positively correlated with the observed expression levels of OsbZIP16 (Chen et al., 2012a). Related data come from studies of GM rice overexpressing

Oshox22, which belongs to the homeodomain-leucine zipper (HD-Zip) family I of transcription factors (Zhang et al., 2012b). These authors conclude that Oshox22 affects ABA biosynthesis and regulates drought and salt responses through ABA-mediated signal transduction pathways. A number of similar results have been reported by overexpression of several diverse genes in GM rice. These include, *OrbHLH001*, a putative helix-loop-helix transcription factor, that confers salt tolerance (Chen et al., 2012a); ZFP182, a TFIIIA-type zinc finger protein, that significantly enhanced multiple abiotic stress tolerances, including salt, cold and drought tolerances (Huang et al., 2012); OsLEA3, a Late Embryogenesis Abundant protein, that showed significantly enhanced growth under saline conditions and was better able to recover after 20 days of drought (Duan and Cai, 2012); a DEAD-box helicase that improves growth in 200mM salt (Gill et al., 2013); and myo-inositol oxygenase (MIOX), (a unique monooxygenase that catalyzes the oxidation of myo-inositol to d-glucuronic acid) that improves drought tolerance by scavenging of reactive oxygenase species (Duan et al., 2012a). Studies on GM rice have also suggested that overexpression of a wheat gene encoding a salt-induced protein (TaSIP) (Du et al., 2013) and a sheepgrass gene (*LcSain1*) (Li et al., 2013e) may also be of benefit in enhancing salt tolerance. An equivalent investigation demonstrated that GM oats expressing the Arabidopsis *CBF3* gene exhibited improved growth and showed significant maintenance of leaf area, chlorophyll content, photosynthetic and transpiration rates, relative water content, as well as increased levels of proline and soluble sugars under high salt stress (Oraby et al., 2012). At a salinity stress level of 100mM, the GM plants showed a yield loss of 4-11% compared with >56% for the non-transgenic control. According to a recent report, field trials conducted in Australia in 2009 (Table 3) showed that wheat lines expressing a salt tolerant gene Nax2) from *Triticum monococcum* produced 25% more yield than the control line in saline conditions (<http://www.isaaa.org/resources/publications/pocketk/document/Doc-Pocket%20K38.pdf>).

381

382 In a similar study two wheat CBF transcription factors, TaCBF14 and TaCBF15, were  
383 transformed into spring barley, and analysis showed that transgenic lines were able to survive  
384 freezing temperatures several degrees lower than that which proved lethal for the wild-type  
385 spring barley (Soltész et al., 2013). Similar results with improved frost tolerance or other  
386 abiotic stress were achieved with GM barley expressing the rice transcription factor *Osmby4*  
387 (Soltész et al., 2011) or the wheat *TaDREB3* gene (Hackenberg et al., 2012; Kovalchuk et al.,  
388 2013).

389

390 Encouraging data have also been produced from studies of GM rice overexpressing OsNAC9,  
391 a member of the rice NAC domain family (Redillas et al., 2012). Root-specific (RCc3) and  
392 constitutive (GOS2) promoters were used to overexpress OsNAC9 and field evaluations over  
393 two seasons showed that grain yields of the RCc3:OsNAC9 and the GOS2:OsNAC9 plants  
394 were increased by 13%-18% and 13%-32% under normal conditions, respectively. Under  
395 drought conditions, RCc3:OsNAC9 plants showed an increased grain yield of 28%-72%.  
396 Both transgenic lines exhibited altered root architecture involving an enlarged stele and  
397 aerenchyma. One approach to the identification of genes that might confer improved drought  
398 tolerance in wheat involves use of the VIGS technique (Manmathan et al., 2013).

399

400 Studies on improving crop growth under conditions of nutritional limitation include results  
401 from the overexpression of *Thellungiella halophila* H<sup>+</sup>-pyrophosphatase gene in maize (Pei et  
402 al., 2012). Under phosphate sufficient conditions, GM plants showed more vigorous root  
403 growth than the wild type, and under phosphate deficit stress they also developed more robust  
404 root systems. This advantage improved phosphate uptake, and the GM plants subsequently  
405 accumulated more phosphorus. In an associated study it was found that overexpression of the

phosphate transporter *Phl1* promoted phosphate uptake in GM rice (Sun et al., 2012). A similar project concerns the use of the phosphate starvation response regulator *Ta-PHR1* to increase yield in wheat (Wang et al., 2013a).

One of the most ambitious of plans to improve growth under conditions of nitrogen deficiency is the project to engineer nitrogen fixation into cereals. For example, the Bill & Melinda Gates Foundation is funding the ENSA (Engineering Nitrogen Symbiosis for Africa) project (<https://www.ensa.ac.uk/news/page/3>).

In addition to the problems of reduced growth under conditions of nutrient deficiency, the ions of certain metals inhibit normal development. One example is the inhibitory effect of excess aluminium in acid soils, and this was the subject of a recent genetic study on the root hairs of wheat (Delhaize et al., 2012). An alternative approach is represented by a study of the multidrug and toxic compound extrusion (*TaMATE1B*) gene in wheat (Tovkach et al., 2013) and in wheat and barley (Zhou et al., 2013). One approach to improving growth in alkaline soils is demonstrated by results from GM rice expressing the barley iron-phytosiderophore transporter (*HvYS1*). This gene enables barley plants to take up iron from alkaline soils, and the GM rice plants grown in alkaline soil exhibited enhanced growth, yield and iron concentration in leaves compared to the wild type plants which were severely stunted (Gómez-Galera et al., 2012).

Other related recent studies include one on GM rice in which overexpression of a protein disulphide isomerase-like protein from the thermophilic archaea *Methanothermobacter thermoautotrophicum* enhances tolerance to mercury (Chen et al., 2012d) and one that

demonstrated the role of the Zn/Cd transporter OSHMA2 in cadmium accumulation in rice (Takahashi et al., 2012).

### 3.5 Yield traits

The obvious aim of all the agronomic traits mentioned to date is to increase or to stabilise yield under field conditions (Shi et al., 2013). There are also future new opportunities to improve the underlying physiological performance of the plant itself. One recent example of this is investigation in rice of the major grain length QTL, qGL3, which encodes a putative protein phosphatase with a Kelch-like repeat domain (OsPPKL1). It was found that a rare allele of this gene, qgl3 leads to a long grain phenotype, and transgenic studies confirmed that OsPPKL1 and OsPPKL3 function as negative regulators of grain length, whereas OsPPKL2 as a positive regulator (Zhang et al., 2012c). Grain size in rice can also be increased by overexpression of a *TIFY* gene, *TIFY11b* (Hakata et al., 2012), whereas grain number in this crop can be increased by expression of the zinc finger transcription factor DROUGHT AND SALT TOLERANCE (DST), which itself regulates the expression of a cytokinin oxidase *Gn1a/OsCKX2* (Grain number 1a/Cytokinin oxidase 2) (Li et al., 2013c). Corresponding transgenic research in wheat has identified the role of TaGW2-A, a functional E3 RING ubiquitin ligase, in regulating grain size (Bednarek et al., 2012).

An important quality trait related to yield is the problem of post harvest sprouting. Among the GM approaches to overcoming this problem is the use of an antisense version of the *trx s* (*thioredoxin s*) gene from *Phalaris coerulescens* to reduce the endogenous *trx h* gene in wheat (Guo et al., 2011).

Amongst the most radical of research efforts are attempts to introduce the C4 photosynthetic trait, as found in maize, into C3 cereals such as rice. This is the subject of many programmes (see C4rice.irri.org). One recent report in this area is the finding that expression of the maize phosphoenolpyruvate carboxylase gene in wheat increases the rate of photosynthesis in the GM plants to 31.95  $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ , some 26% greater than the rate in untransformed control plants (Hu et al., 2012c). It was also found recently that constitutive expression of the rice gene OsTLP27 under the control of the CaMV 35S promoter resulted in increased pigment content and enhanced photochemical efficiency in terms of the values of maximal photochemical efficiency of photosystem II (PSII) ( $F(v)/F(m)$ ), effective quantum yield of PSII ( $\Phi\text{PSII}$ ), electron transport rate (ETR) and photochemical quenching (qP) (Hu et al., 2012a).

Of course, in any studies of GM cereals, as with other crops, it is always important to examine the whole plant performance, including the photosynthetic efficiency, in order to identify any non-intended effects (Sun et al., 2013).

## 4 Output traits

### *4.1. Modified grain quality*

#### *4.1.1. Nutrition*

Transgenic technologies provide a large variety of opportunities to modify the nutritional components in cereal crops (Bhullar and Grissem, 2013; Demont and Stein, 2013; Morell, 2012; Pérez-Massot et al., 2013; Rawat et al., 2013). These include modified proteins



(Wenefrida et al., 2013), carbohydrate, oils, and other minor compounds and these will be considered in turn.

Among the first reported GM lines of wheat were ones with modified subunits of the high molecular weight glutenin protein that confers good breadmaking quality. Recent reports in this area include the generation of GM wheat with enhancement in the concentration of high-molecular-weight glutenin subunit 1Dy10 and associated benefit in sponge and dough baking of wheat flour blends (Graybosch et al., 2013). It is also reported that such improved baking quality can be achieved without the need for selectable marker genes (Qin et al., 2013), and that coexpression of high molecular weight glutenin subunit 1Ax1 and puroindoline improves dough mixing properties in durum wheat (*Triticum turgidum* L. ssp. *durum*) (Li et al., 2012b). Similarly it is reported that GM methods can be used to reduce the expression of  $\gamma$ -gliadins and thereby potentially improve the dough mixing and bread making properties of wheat flour (Gil-Humanes et al., 2012). As part of related projects it has been shown that the starch characteristics of GM wheat overexpressing the Dx5 high molecular weight glutenin subunit are substantially equivalent to those in nonmodified wheat (Beckles et al., 2012), and that isolation of enriched gluten fractions from lines modified to overproduce HMW glutenin subunits Dx5 and/or Dy10 may require modified separation technologies (Robertson et al., 2013). Studies on the GM modification of such subunits may also lead to the production of novel proteins encoded by altered versions of either the transforming or endogenous genes (Blechl and Vensel, 2013). A relevant similar study is that on transgenic rice seed expressing the wheat HMW subunit (Oszvald et al., 2013). Another aspect of this type of study that has importance in any future regulatory submission is the determination of potential changes in the allergenicity of the GM material (Lupi et al., 2013).

In addition to efforts to modify baking and bread-making quality there have also been projects to modify the particular amino acid profile of cereals, in particular to increase the levels of lysine. GM approaches in this area have included the expression of the *sb401* gene, which encodes a lysine-rich protein, in GM maize; this leads to increased levels of lysine and total protein in the seeds (Tang et al., 2013) (see also Wang et al., 2013c). A three generation rat feeding trial of GM rice with increased levels of lysine has shown no adverse effects (Zhou et al., 2012a). In a related study, expression of a bacterial serine acetyltransferase (EcSAT) in rice lead to significantly higher levels of both soluble and protein-bound methionine, isoleucine, cysteine, and glutathione (Nguyen et al., 2012).

Alongside the many projects that are designed to modify protein quantity and quality in cereals are several that focus on aspects of starch synthesis (Blennow et al., 2013). These include GM rice lines produced by introducing a cDNA for *starch synthase IIa* (*SSIIa*) from an indica cultivar (*SSIIa* (I), coding for active *SSIIa*) into an isoamylase1 (*ISA1*)-deficient mutant (*isa1*) that was derived from a japonica cultivar (bearing inactive *SSIIa* proteins). The storage  $\alpha$ -glucan of these GM lines was shown to have altered solubility and crystallinity (Fujita et al., 2012). Many of these projects are designed to produce products with improved health benefits. For example, using a chimeric RNAi hairpin Carciofi et al. (2012a) simultaneously suppressed all genes coding for starch branching enzymes (SBE I, SBE IIa, SBE IIb) in barley, resulting in production of amylose-only starch granules in the endosperm. The authors claim that this is the first time that pure amylose has been generated with high yield in a living organism, and the resulting lines with so-called “resistant starch” would have potential in reducing the glycaemic index of diets. Such improvements may be of particular value to diabetics and this has been shown experimentally in a study in which a high-amylose GM rice, produced by inhibition of two isoforms of the starch branching enzyme, improved

indices of animal health in normal and diabetic rats (Zhu et al., 2012). It was observed in a similar study on GM durum wheat, in which the gene encoding one isoform of SBE was silenced, that various protein differences were present in the endosperm of the transgenics (Sestili et al., 2013). Rapid testing of constructs for use in such studies may be achieved by using transgenic callus, rather than mature seed; this system has been developed first in barley (Carciofi et al., 2012b).

GM triticale lines expressing one or both of the *sucrose-sucrose 1-fructosyltransferase (1-SST)* gene from rye and or the *sucrose-fructan 6-fructosyltransferase (6-SFT)* gene from wheat accumulated 50% less starch and 10-20 times more fructan, particularly 6-kestose, in the dry seed compared to the untransformed control (Diedhiou et al., 2012). This is one of the first reports of GM cereals with production of fructans (Kooiker et al., 2013) in seeds.

An alternative route to the alteration of starch content was demonstrated by a study on GM maize expressing the potato gene *StSUS* that encodes an isoform of sucrose synthase. Seeds from these transgenic plants accumulated 10-15% more starch at the mature stage, and contained a higher amylose/amylopectin balance than the WT control seeds (Li et al., 2013a). Possibly the most complex of these studies on maize was that in which the expression of six genes was modified; this led to a 2.8-7.7% increase in endosperm starch and a 37.8-43.7% increase in the proportion of amylose (Jiang et al., 2013a). Additionally there was a 20.1-34.7% increase in 1000-grain weight and a 13.9-19.05% increase in ear weight. Other associated studies include the effect of the granule-bound starch synthase (GBSS), (known as waxy protein), on the amylose content of GM durum wheat (Sestili et al., 2012).

Among other investigations of starch biosynthetic pathway is that on the maize *shrunk-2* (*Sh2*) gene, which encodes the large subunit of the rate-limiting starch biosynthetic enzyme, ADP-glucose pyrophosphorylase (Tuncel and Okita, 2013). Expression in maize of a transgenic form of this enzyme with enhanced heat stability and reduced phosphate inhibition was shown to increase yield up to 64% (Hannah et al., 2012). The extent of this yield increase was found to be dependent on temperatures during the first 4 days post pollination, and the authors also demonstrated that the transgene acts in the maternal tissue to increase seed number, and thus yield.

Suppression of the *CSLF6* gene in wheat has been shown to reduce the level of glucan and provides an opportunity to improve the level of dietary fibre (Nemeth et al., 2010), and similar suppression of glucosyl transferase genes decreases the arabinoxylan content (Lovegrove et al., 2013).

GM wheat and barley with a range of modified grain traits are among the list of lines that have been tested in the field in Australia (Table 3).

In the area of lipid research it has been shown that the levels of oleic acid (Zaplin et al., 2013) and  $\alpha$ -linolenic acid (Liu et al., 2012) in rice seed can be increased by manipulation of various fatty acid desaturase (FAD) genes.

Another significant area relates to vitamin and mineral content, particularly iron, with studies on rice and maize summarised in Table 4. The classic example of vitamin increase is the generation of “Golden Rice” (Potrykus, 2012) with higher levels of provitamin A, a compound deficient in many subsistence diets based on rice. Such deficiency may lead to

juvenile blindness and even death. Other recent results on modifying vitamin levels in rice include expression of *Arabidopsis thaliana*  $\rho$ -hydroxyphenylpyruvate dioxygenase (HPPD), which catalyzes the first committed step in vitamin E biosynthesis (Farré et al., 2012, 2013) and *Arabidopsis*  $\gamma$ -tocopherol methyltransferase ( $\gamma$ -TMT) (Zhang et al., 2013a), which catalyzes the final step in this pathway. In a related study, Chaudhary and Khurana (2013) produced GM wheat overexpressing the endogenous *HPPD* gene and observed a 2.4 fold increase in the level of tocochromomanol, one of an important group of plastidic lipophilic antioxidants, which may have significant benefits in the human diet.

Results relating to iron and zinc accumulation in GM wheat expressing a ferritin gene have been discussed recently by Neal et al. (2013). In addition to increases in the levels of vitamins and minerals, GM techniques have also been used recently to improve the content of beneficial compounds such as flavonoids (Ogo et al., 2013) and sakuranetin, a flavonoid phytoalexin (Shimizu et al., 2012a) in rice. Related research demonstrating the effects of purple, anthocyanin-containing, wheat on extending the lifespan of nematodes (Chen et al., 2013b) may be developed through GM technology.

#### 4.2 Enzymes, diagnostics and vaccines

Probably the first commercial plant –derived industrial enzyme was trypsin, produced in maize kernels and marketed by Sigma (Product Code T3449) under the brand name TrypZean®. This company also markets maize-derived recombinant avidin (Product Code A8706). As summarised recently (Xu et al., 2012b) other recombinant products produced from corn included  $\beta$ -glucuronidase, aprotinin and a range of degradative enzymes (also see

biofuel section below). There have been significant environmental concerns expressed in the USA with some of these plant derived products.

Among the most significant of GM maize products are those expressing the phytase enzyme. Such products are designed to overcome the problem caused by phytate, a phosphorus containing compound that is present in maize grain but one in which the phosphate is unavailable to monogastric animals such as poultry and pigs and therefore causes pollution from their waste. Maize expressing a phytase gene from *Aspergillus niger* is the first GM maize to receive a biosafety certificate in China (Chen et al., 2013a) (see also Xia et al., 2012). An alternative approach is to use RNAi techniques to downregulate the *myo*-inositol-3-phosphate synthase (*MIPS*) gene that catalyzes the first step of phytic acid biosynthesis in rice (Ali et al., 2013), or to employ cisgenic methods (Holme et al., 2012b). The value of such low-phytate maize products has been recently confirmed in feeding trials with poultry (Gao et al., 2012; Ma et al., 2013; Wang et al., 2013e) and pigs (Li et al., 2013d). A similar benefit may derive from GM maize expressing a fungal  $\beta$ -mannanase from *Bispora* (Xu et al., 2013b).

Although no GM lines in this category have yet been approved for commercialisation, there has been considerable activity, over many years, in the area of plant-derived vaccines and other potential pharmaceutical products. This summary describes some of the recent activity in this 'pharming' area. The justification for such research lies in the assumed economic benefit that might derive from using plants rather than other expression systems (eg animal cells or bacteria) for production of high-value, bioactive compounds. Cereals, principally rice (Greenham and Altosaar, 2012; Takaiwa, 2013), maize, and barley (Magnusdottir et al., 2013) (<http://www.orfgenetics.com/>) have become the crops of choice, as proteins can be

expressed at high levels in the seed and stored for extended periods without significant deterioration. Additionally, seed-derived antigens provide the possibility of oral delivery as an alternative to injection; this method may be of particular relevance in the area of veterinary medicine. Recent examples include the induction of a protective immune response to rabies virus in sheep after oral immunization with GM maize kernels that express the rabies virus glycoprotein (Loza-Rubio et al., 2012), and the proven immunogenicity of foot-and-mouth disease virus structural polyprotein P1 (Wang et al., 2012) and MOMP protein (Zhang et al., 2013a) expressed in GM rice, and the porcine reproductive and respiratory syndrome virus (PRRSV) expressed in GM maize (Hu et al., 2012b). Other similar examples are the demonstration of immunogenicity of a neutralizing epitope from porcine epidemic diarrhoea virus (PEDV) fused to an M cell-targeting ligand fusion protein and expressed in GM rice (Huy et al., 2012) and the successful production of the hepatitis B surface antigen (HBsAG) in maize (Hayden et al., 2012a,b). This latter study represents the first description of a commercially feasible oral subunit vaccine production system for a major human disease, though there has also been much publicity given to the potential of maize as a production system for an HIV neutralizing monoclonal antibody (Sabalza et al., 2012).

Recently it was confirmed that rice-derived recombinant human serum transferrin (hTF) represents a safe and animal-free alternative to human plasma-derived hTF for bioprocessing and biopharmaceutical applications (Zhang et al., 2012).

Another area of related research is that on allergens. For example, GM rice seeds have been used for the production of a recombinant hypoallergenic birch pollen allergen Bet v 1 (Wang et al., 2013d), and a hypoallergenic Der f 2 (Yang et al., 2012a) and Der p 1 (Saeki et al., 2012, 2013) derivatives of the House Dust Mite (HDM) allergen from *Dermatophagoides*

*pteronysinus*. These products may be useful in allergen-specific immunotherapy. Similarly, human interleukin IL-10 (hIL-10), a therapeutic treatment candidate for inflammatory allergy and autoimmune diseases, has been produced in rice seed and effectively delivered directly to gut-associated lymphoreticular tissue (GALT) via bio-encapsulation (Yang et al., 2012b). Related research is being conducted on the similar molecule hIL-7 (Kudo et al., 2013). Rice is also the production system for human alpha-antitrypsin (AAT), a compound used as therapy of individuals with mutations in the *AAT* gene (Zhang et al., 2013b).

### 4.3 Biofuels

To date the only GM cereal with a biofuel-related trait that has been commercialised is Enogen™, a maize hybrid expressing a thermostable alpha amylase for efficient starch hydrolysis and higher bioethanol yields. Details of this Syngenta product, which was approved by the USDA on 12<sup>th</sup> February 2011, are available at (<http://www.syngenta.com/country/us/en/enogen/Pages/Home.aspx> and <http://www.syngenta.com/country/us/en/agriculture/seeds/corn/enogen/stewardship/Documents/June%2014th,%202011/Enogen%20Overview.pdf>). It is stated that ethanol throughput during fermentation with this product is increased by 5.2% and the financial benefit is between 8-15 US cents per gallon. A news item from 12<sup>th</sup> June 2013 (<http://www.agprofessional.com/news/Syngenta-footprint-for-Enogen-corn-grows-to-11-ethanol-plants-211053531.html>) states that a total of 11 ethanol plants in the US have now signed agreements to use this product; such plants pay the farmer an average premium of 40 cents per bushel for Enogen™ corn. Present research in Syngenta and elsewhere is also focussed on the potential for the production of recombinant cell-wall degrading enzymes in GM plants, in order to avoid the significant cost of adding exogenous enzymes during the



production of fermentable sugars from biomass (Sainz, 2009). As part of this strategic goal, Syngenta have signed research agreements which include those with Diversa in 2007, and Verenum (now owners of Diversa) and Protéus in 2009.

Other relevant recent studies in this area include the production of:- bacterial amylopullulanase in maize grain (Nahampun et al., 2013); thermostable xylanase in maize stover (Shen et al., 2012); glycoside hydrolases (Brunecky et al., 2012); and an *Acidothermus cellulolyticus* endoglucanase in transgenic rice seeds (Zhang et al., 2012a). Additionally, down regulation of the enzyme cinnamyl alcohol dehydrogenase in maize has been shown to produce a higher amount of biomass and a higher level of cellulosic ethanol in assays (Fornalé et al., 2012). It is hoped that these various approaches will lead to significant improvements in the efficiency of biofuel production and thereby reduce the conflict between the demands for food and fuel (Zhang, 2013).

## **5 Pipeline of future products**

### *5.1 Field trials*

One simple method to assess the direction of future research on GM cereals in both commercial and non-commercial programmes is to examine the various public databases that summarise the applications for field testing. Such information is available from the regulatory authorities in the various jurisdictions around the world. Data for the USA are available at <http://www.isb.vt.edu/search-release-data.aspx> and can be summarised as follows:-

**Maize:** A total of 8294 applications have been submitted in the period from 1996 to date (latest 14<sup>th</sup> June 2013). Many of these are from commercial companies and understandably have limited details of the genes being tested because of Confidential Business Information (CBI) restrictions. However, among the most recent application from a non-commercial institution is one from the Cold Spring Harbor Laboratory that lists a total of 78 genes to be tested.

**Wheat:** A total of 510 applications for have been submitted in the period from 1996 to date (latest 22<sup>nd</sup> April 2013). The traits for trial in the 13 applications for 2013 include:- Nitrogen use efficiency (Arcadia); Fusarium resistance (Uni. Minnesota); nitrogen metabolism, drought/heat tolerance, water use efficiency, yield increase, modified flowering time, altered oil content, fungal tolerance, insect resistance, herbicide tolerance (Monsanto); increased carbohydrate, improved grain processing (Uni. Nebraska); herbicide tolerance (and other CBI traits) (Pioneer); and CBI traits (Biogemma); breadmaking quality (USDA).

**Barley:** a total of 109 applications were submitted in the period from 1994 to 2013 (latest 15<sup>th</sup> May 2013). The traits for trial in the 6 applications for 2012 include:- starch quality (USDA); nitrogen utilisation efficiency (Arcadia); Fusarium resistance (USDA); and Rhizoctonia resistance (Washington State University).

Data for the EU are available at [http://gmoinfo.jrc.ec.europa.eu/gmp\\_browse.aspx](http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx) and are summarised in Table 5. This list is relatively short and does not include many of the commercial trials of maize. Among the interesting trials is that testing wheat designed to have reduced levels of epitopes linked to celiac disease, and that designed to deter aphids by expression of an alarm pheromone.

Data from Australia are available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>. A summary is given in Table 3, which identifies trials of wheat and barley with modified grain traits and with various genes providing tolerance to abiotic stress. More complete detail may be obtained from the application dossiers published by the various regulatory authorities.

## 5.2 Patents

In any consideration of future trends it is of great value to assess the patent literature, as this provides a summary of those novel technologies that are the subject of research activity, particularly in commercial companies who will publish information in patent applications prior to it emerging in the conventional scientific literature. The most recent overall review of this area is that of Dunwell (2010) who includes a discussion of IPR relevant to the research scientist and to those interested in international development, globalization, and sociological and ethical aspects of the public- and private-sector relationships. Data on patent application and granted patents are available in many publically accessible databases, with the most complete being that at <http://www.patentlens.net/>. The extent of patent activity in the area of GM cereals is exemplified by the selection of recent US patents (Table 6a) and patent applications (Table 6b). The subject matter of these patents, taken from a short period of time, covers all the major themes discussed in this review. It is always necessary to point out the commercial reality that few, if any, of the patents and applications in these lists will ever produce a financial profit. The most common reasons for this lack of success are unexpected additional costs of development or failure of the underlying science during the transfer from laboratory to field scale.

### 5.3 New Breeding Techniques

It is more than twenty years ago that the various GM regulatory legislations were enacted. For example, the first iteration of the EU Directive that controls the Deliberate Release of genetically modified organisms (GMOs) into the environment was adopted in 1990. The foundation of this approach was to define an organism based on how it was made and the nature of the resulting alterations to its genetic material. However, since that time a number of reports, including the last review of the current 2001/18 Directive (EPEC, 2011), have highlighted concerns about the clarity of the definition of a GMO when applying it to organisms produced by particular new methodologies. These new breeding techniques (NBTs) include: cisgenesis/intragenesis; site directed mutagenesis; genome editing using zinc finger nucleases, TALENs (Wendt et al., 2013), CRISPRs (Shan et al., 2013) and other similar systems (Li et al., 2013b; Nekrasov et al., 2013); RNA dependent DNA methylation (and other epigenetic methods) (Higo et al., 2012), and reverse breeding. Reports that have considered these NBTs in more detail include that from an EU Commission Working Group on 'New Techniques', a series of papers by the Dutch committee COGEM (COGEM, 2006, 2009, 2010) and an Austrian report (Brüller et al., 2012). A report from the EU Joint Research Centre also provides useful background on the subject (Lusser et al., 2011). In principle, these techniques can be applied to any crop, including cereals. For example, there is much support in certain areas for the concept of cisgenesis, whereby the DNA introduced into recipient crop comes from a sexually compatible relative, and this method has been used to produce low-phytate barley (Holme et al., 2013). In some of these methods, although molecular gene transfer techniques are used to generate the new line, there is no transgene present in the final product. Example of this involve techniques for the modification of recombination or the rapid generation of mutants

by suppressing the activity of DNA repair systems (Xu et al., 2012c) or generating transposon induced chromosomal rearrangements (Yu et al., 2012).

Such problems of enforcement and uncertainty about whether or not new methods fall within the existing legislation (Pauwels et al., 2013) has led many to argue in favour of a so-called “phenotype” (or “product”) based (EASAC, 2013) or “process-agnostic” system (Ammann, 2013).

## **6 Acceptance of GM crops**

The commercial exploitation of GM crops varies greatly across the globe with a clear dichotomy between the position in North and South America, where such crops are grown widely, to Europe where there is little GM agriculture, though large imports of GM material for animal feed (Fresco, 2013; Masip et al., 2013). The foundation for this difference lies in a complex mixture of political, social and economic considerations. Within Europe it has been argued by some that the present regulatory impasse, whereby it has not proved possible for the 29 EU states to achieve political consensus for approval of GM crops for cultivation, should be bypassed by allowing states to determine their own policy. However, others consider this to a retrogressive approach that would lead to dangerous inconsistencies in the regulatory approach (Biszko, 2012).

### *6.1 Regulatory aspects*

Before any GM product can reach the market it must receive approval from the relevant regulatory authority in the appropriate legislative area. The two most important aspects of such a process are food and feed safety and the potential for harm to human health and the environment (Romeis et al., 2013). There is great deal of published information on these topics (eg <http://www.efsa.europa.eu/en/panels/gmo.htm>) and it will not be repeated here, but some of the recent information on compositional analysis has been summarised by Herman and Price (2013), Kitta (2013) and Privalle et al. (2013). Other specific recent data include information on transcriptome changes in maize expressing a phytase gene (Rao et al., 2013), tests for possible changes in allergens in GM maize (Fonseca et al., 2012) and a proteomic study on GM rice (Gong et al., 2102). Animal feeding tests (Buzoianu et al., 2013) are also a required part of any regulatory process, though the outcome of some such tests has recently provoked further controversy about GM safety (Arjó et al., 2013; Fresco, 2013) .

As regards possible environmental effects, a large-scale analysis has shown convincing evidence that one consequence of the global cultivation of GM crops has been a significant reduction both in the amount of pesticide sprayed (~8-9%) and in the release of greenhouse gas emissions from the cropping area (Brookes and Barfoot, 2013b).

Other environmental issues with all GM crops include possible transgene spread to wild relatives (Chandler and Dunwell, 2008). Among the important variables in this context is the relative fitness of the crop-weed hybrid and this is the subject of a recent study that examined GM insect resistant rice (Yang et al., 2012c). Recent studies on GM wheat include assessment of the impact of any GM pollen transfer either within or between crops (Loureiro et al., 2012; Foetzki et al., 2012; Rieben et al., 2011). There is also discussion about the possible persistence of feral populations of GM crops (Raybould et al., 2013).

819

820 An interesting additional aspect relates to the possible effect of GM crops on the soil  
 821 microflora. This is the subject of one study on rice in which the expression of phenylalanine  
 822 ammonia-lyase was inhibited by RNAi methods (Fang et al., 2013). It was concluded that the  
 823 GM rice had less rhizospheric bacterial diversity than the non-GM control.

824

## 825 *6.2 Public perception*

826 This is a very complex area and there have been many published surveys on consumer  
 827 attitudes to GM. Some of these surveys are international in scope (Frewer et al., 2013)  
 828 whereas others examine attitudes in specific regions such as Europe (Cecciolli and Hixon,  
 829 2012; Gaskell et al., 2011), Switzerland (Speiser et al., 2013), Spain (Costa-Font and Gil,  
 830 2012; Rodríguez-Entrena and Sayadi, 2013) and Japan (Ishiyama et al., 2012). Among issues  
 831 considered in such surveys are questions relating to basic knowledge of science (Mielby et  
 832 al., 2013), ethics (Du, 2012; Gregorowius et al., 2012), human rights (Srivastava, 2013),  
 833 effects on the developing world (Jacobsen and Myhr, 2013; Okeno et al., 2012), the need for  
 834 choice (Mather et al., 2012), labelling (Benny, 2012), and coexistence with organic  
 835 agriculture (Areal et al., 2012).

836

## 837 **7 Conclusions**

838

839 It remains to be seen whether the prospects and opportunities (Chen and Lin, 2013; Dunwell,  
 840 2011) described above will be translated into successful GM products in the future and  
 841 whether GM technologies are compatible with sustainable (Bruce, 2012; Hansson and  
 842 Joelsson, 2012) and biodiverse (Jacobsen et al., 2013) agriculture.

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844 **References**

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Table 1. Global area, production, yield and contribution to the human diet for major cereal crops

	2010 (FAOSTAT)					2009 (FAOSTAT)			
	Area		Production		Yield	Energy		Protein	
	Mha	%	MT	%	Tonnes/ha	kcal/ capita/d	%	g/ capita/d	%
Wheat	217	32	651	27	3.0	532	18.8	16.2	20.4
Maize	162	24	844	35	5.2	141	5.0	3.4	2.3
Rice	154	23	672	28	4.4	536	18.9	10.1	12.7
Barley	48	7	123	5	2.6	7	0.2	0.2	0.3
Sorghum	41	6	56	2	1.4	32	1.1	1.0	1.3
Total	683	100	2432	100	3.6	1248	44	30.9	38.6

Adapted from Wheat Initiative (2013)

Table 2. Evolution of wheat yield over 10-year periods since 1960 (FAO) and projected needs for 2050

Period	Mean area harvested/yr (Mha)	Mean production/yr (Mt)	Mean production increase/yr (%)	Mean yield (t/ha)	Mean yield increase/yr (%)
1961-1970	213	278		1.3	
1971-1980	225	388	3.9	1.7	3.2
1981-1990	229	509	3.1	2.2	2.9
1991-2000	220	571	1.2	2.6	1.7
2001-2010	216	622	0.9	2.9	1.1
2050 (target)	220	1045	1.7	4.75	1.6

Adapted from Wheat Initiative (2013)

Table 3. Field trials of GM wheat and barley in Australia: Applications and licences for Dealings involving Intentional Release (DIR) into the environment

Number	Organisation	Description	Crop(s)	Trait	Date
DIR117	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2013
DIR112	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2012
DIR111	CSIRO	grain composition, nutrient utilisation	wheat, barley	yield, disease, stress	Feb 2012
DIR102	Uni. Adelaide	abiotic stress	wheat, barley	yield, stress	Jun 2010
DIR100	CSIRO	drought, heat	wheat	yield, stress	Jun 2010
DIR099	CSIRO	grain composition, nutrient utilisation	wheat, barley	nutrition, yield	Mar 2013
DIR094	CSIRO	nutrient utilisation	wheat, barley	yield	Jul 2009
DIR093	CSIRO	grain starch	wheat, barley	nutrition	Jun 2009

DIR092	CSIRO	grain composition	wheat	nutrition, processing	May 2009
DIR080	Vict. Dept.  Prim. Indust.	drought	wheat	abiotic stress	Jun 2008
DIR077	Uni. Adelaide	stress, glucan	wheat, barley	stress, nutrition	Jun 2008
DIR071	Vict. Dept.  Prim. Indust.	drought	wheat	abiotic stress	Jun 2007
DIR061	Grain Biotech	salt tolerance	wheat	stress tolerance	Withdrawn
DIR054	CSIRO	grain starch	wheat	nutrition	Apr 2005
DIR054	Grain Biotech	salt tolerance	wheat	stress tolerance	Apr 2005

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Summary of data from the Office of the Gene Regulator. Available at:-

<http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/ir-1>

Table 4. Transgenic cereals with enhanced content of vitamins and minerals

Nutrient	Species	Genes used	Total increase (fold increase over WT)	References
Vitamin A	Maize	<i>PacrtB, PacrtI</i>	33.6 µg/g DW (34)	Aluru et al., 2008
	Maize	<i>Zmpsy1, PacrtI, PcrW, Gllycb</i>	146.7 µg/g DW (133)	Zhu et al., 2008
	Maize	<i>Zmpsy1, PacrtI</i>	163.2 µg/g DW (112)	Naqvi et al., 2009
	Wheat	<i>Zmpsy1, PacrtI</i>	4.96 µg/g DW (10.8)	Cong et al., 2009
	Rice	<i>Nppsy1, EucrI</i>	1.6 µg/g	Ye et al., 2000
	Rice	<i>Zmppsyl, EucrI</i>	37 µg/g (23)	Paine et al., 2005
Vitamin C	Maize	<i>Osdhar</i>	110 µg/g DW (6)	Naqvi et al., 2009
Vitamin E	Rice	<i>HPPD</i>		Farré et al., 2012
		$\gamma$ -TMT		Zhang et al., 2013a
Folic acid	Rice	<i>Atgtpchi, Atadcs</i>	38.3 nmol/g (100)	Storozhenko et al., 2007
Iron	Rice	<i>Osnas2</i>	19 µg/g DW in polished seeds (4.2)	Johnson et al., 2011
	Rice	<i>Gm ferritin, Af phytase, Osnas1</i>	7 µg/g DW in polished seeds (4–6.3)	Wirth et al., 2009
	Rice	Activation tagging of <i>Osnas3</i>	32 µg/g DW in dehusked seeds (2.9)	Lee et al., 2009

Zinc	Maize	<i>Gm ferritin</i> and <i>Af phytase</i>	30 µg/g DW in whole seed (2)	Drakakaki et al., 2005
	Rice	<i>Ferritin</i>	7 µg/g DW in polished seed (6)	Masuda et al., 2012, 2013
	Rice	Activation tagging of <i>Osnas2</i>	40–45 µg/g DW in polished seeds (2.9)	Lee et al., 2011
	Rice	<i>Osnas2</i>	52–76 µg/g DW in polished seeds (2.2)	Johnson et al., 2011
	Rice	<i>Gm ferritin</i> , <i>Af phytase</i> , <i>Osnas1</i>	35 µg/g DW in polished seeds (1.6)	Wirth et al., 2009

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Data adapted from Pérez-Massot et al. (2012) and other sources.



Table 5. Summary of selected field trials of GM cereals in the EU

Number	State	Date	Institution	Subject
B/ES/13/19	Spain	May 2013	INIA	Bt maize
B/ES/13/20	Spain	May 2013	CSIC	Wheat with low content of celiac-toxic epitopes
B/ES/13/15	Spain	March 2013	Limagrain	Bt, HR maize
B/ES/13/16	Spain	March 2013	Uni. Lleida	High vitamin maize
B/DK/12/01	Denmark	April 2012	Univ. Aarhus	Cisgenic barley with improved phytase activity
B/SE/12/484	Sweden	Feb 2012	Swedish Univ. Agric. Sci.	Barley with improved nitrogen use efficiency
B/GB/11/R8/01	UK	Oct 2011	Rothamsted	Wheat producing aphid alarm
B/PL/11/02-10	Poland	Sept 2011	Plant Breed. Acclim. Instit.	Transgenic Triticale
B/CZ/11/2	Czech	Mar 2011	Instit. Exper. Botany	Barley with phytase
B/IS/09/01	Iceland	Apr 2009	ORF Genetics	Transgenic barley, comparison

of processing quality

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Available from JRC database ([http://gmoinfo.jrc.ec.europa.eu/gmp\\_browse.aspx](http://gmoinfo.jrc.ec.europa.eu/gmp_browse.aspx))

Table 6. Summary of selected USA granted patents (a) and patent applications (b) relating to GM cereals; data from 2013. Data are from the USPTO (<http://www.uspto.gov/patents/process/search/index.jsp>).

(a)

Number	Date	Inventor	Subject
8,440,886	14 May	Lundquist et al.	Transgenic maize
8,440,881	14 May	Park et al.	Genes for yield
8,431,775	30 April	Hegstad et al.	<i>knotted1</i> gene
8,431,402	30 April	Vasudevan et al.	Sorghum regeneration
8,426,704	23 April	Hirel et al.	Glutamine synthetase
8,426,677	23 April	Yu et al.	GA20 oxidase
8,426,676	23 April	Oswald et al.	Pyruvate kinases
8,420,893	16 April	Gordon-Kamm et al.	AP2 domain transcript. factor
8,415,526	9 April	McGonigle	Artificial microRNAs
8,404,933	26 March	Chen et al.	Herbicide resistance gene
8,404,930	26 March	Wu et al.	Monocot transformation
8,404,929	26 March	Gruis et al.	Reducing gene expression

(b)

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20130133111	23 May	Lyznik et al.	MAPKKK genes to improve yield
20130133101	23 May	Rodiuc et al	Phytosulfokines and pathogen resistance
20130125266	16 May	Hiei et al.	Agrobacterium, barley transformation
20130125264	16 May	Frankard et al.	Genes for yield
20130125258	16 May	Emmanuel et al.	Genes for yield
20130117894	9 May	Frohberg et al.	Starch synthase
20130117888	9 May	Sanz Molinero et al.	Genes for yield
20130116124	9 May	Fernandez et al.	Bacterial volatiles and starch
20130111634	2 May	Kurek et al.	Artificial microRNAs
20130111632	2 May	Champion et al.	Jasmonic acid
20130111620	2 May	D'Halluin et al.	Meganucleases
20130111618	2 May	Mankin et al.	Herbicide tolerance

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